CERTIFICATE OF TRANSMISSION
I hereby certify that this paper is being facsimile transmitted to the attention of Examiner Elizabeth Slobodyansky, Group Art Unit 1652, USPTO, at Facsimile No. 703-308-4242 on 3 September 2002.

Lynn E. Murry

Signature of Lynn E. Murry

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Lal et al.		
Title:	**HUMAN REGULATORY MOLECULES**		
Serial No.:	09/840,787	Filing Date:	23 April 2001
Examiner:	Slobodyansky, E.	Group Art Unit	1652

Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 CFR 1.132 OF PREETI G. LAL

I, Preeti G. Lal, declare:

1. I received my BS and MS degrees in Microbiology from Bombay University in 1983 and 1985, respectively. I received my PhD in Microbiology and Immunology from the Medical University of South Carolina in Charleston, South Carolina in 1993. I did postdoctoral work at Stanford University Department of Pathology from 1994-1995. I was hired at Incyte Genomics in 1995 and have worked as a Research Scientist in Genomics and in Protein Biology for the past seven years. My work has involved the analysis, characterization and annotation of molecules by their homology and expression in relation to cellular function, disease, and metabolic pathways.

2. The above-identified application relates to a novel human regulatory molecule that is useful in the diagnosis of diseases such as cancer. I am the first inventor on this application.

3. I understand that an OFFICE ACTION was mailed by the USPTO on May 1, 2002, and in that office action, the Examiner rejected claims 2-14 under 35 USC §§ 101 and 112 directed to the described invention as "not supported by either a specific asserted utility or a well established utility".

4. The purpose of my declaration is to substantiate the characterization of the molecule at the time of filing by motif and alignment as a mitochondrial carrier protein and the asserted utility of the cDNA encoding SEQ ID NO:19 (i.e. SEQ ID NO:68) as a cancer diagnostic, especially for lung cancer. To that end, I have attached the following Exhibits:

    (A) A copy of an article by Yu et al. (2001; Overexpression of the human 2-oxoglutarate carrier lowers mitochondrial membrane potential in HEK-293 cells: contrast with the unique cold-induced mitochondrial carrier CGI-69. Biochem J 353:369-375);

(B) A Clustal W alignment of HRM-19 with the known human mitochondrial carriers CGI-69 protein (GenBank accession no. AAD34064), mitochondrial carrier protein CGI-69 long form (GenBank accession no. AAD34064), and 2-oxoglutarate carrier protein (GenBank accession no. AAC28637);

(C) GenBank reports for the mitochondrial carriers in the **Exhibit B** alignment; and

(D) Lung microarray data.

5. I will now summarize the relevant points of the Yu article (**Exhibit A**) that classify HRM-19 as a mitochondrial carrier protein, specifically as one of the members of the CGI-69 mitochondrial carrier protein family.

First, Yu states that he isolated "a variety of human CGI-69" clones from liver (see left column, bottom of page 371 of the Yu article) including ones that encode proteins matching:

- CGI-69 (AF151827 dated 18 May, 2000 in GenBank),
- CGI-69_{L} (AF317711 dated 13 Dec, 2001 in GenBank), a "longer" molecule containing an eight amino acid insert preceded by a W64 $\rightarrow$ L substitution and an F247 $\rightarrow$ L substitution; and,
- $\text{CGI-69}_{F239\rightarrow L}$, a molecule matching CGI-69 but containing an F239 $\rightarrow$ L substitution.

Note: This last molecule is identical to HRM-19 (SEQ ID NO:19) of the claimed invention.

Second, analysis of the CGI-69 protein structure indicated the presence of four mitochondrial carrier domains, six potential transmembrane spanning regions, a likely mitochondrial localization motif and three regions with reasonable homology to putative mitochondrial energy-transfer signature motifs present in known uncoupling protein functional family members (See Figure 2 of the Yu article).

Finally, please note that Yu purchased a full-length human 2-oxoglutarate carrier (OGC) cDNA clone from Incyte Pharmaceuticals and subcloned it into an expression vector. The Incyte clone used by Yu encodes the most abundant form of OGC in humans. (See lines 8-11 of the right column of page 370 of the Yu article).

6. I have obtained coding sequences for the mitochondrial carrier proteins mentioned above from GenBank (provided as **Exhibit C**) and used the CLUSTAL W algorithm to align these sequences and obtain comparison scores. Exhibit B shows the identity between the cDNA encoding HRM-19 and three human mitochondrial carrier proteins. The alignment scores (Sequences (1:2) Aligned. Score: 99 and Sequences (1:3) Aligned. Score: 99) and conserved mitochondrial energy-transfer signature motifs (shown in bold confirm that the Incyte cDNA encoding HRM-19 is known in the art as mitochondrial carrier protein. The mitochondrial carrier motif, P$_{31}$LDVVKVRL, was described on page 18, line 27, of the specification at the time of filing.

7. I will now present evidence illustrating that HRM-19 and its encoding polynucleotide can be used in the detection of lung cancer. One way of demonstrating this utility is to show the expression profiles for transcripts that encode HRM-19. This analysis has been done for SEQ ID NO:68 employing microarray analysis methods known in the art. The analysis was done on tissue samples obtained from patients with lung cancer where <u>both tumor and cytologically normal samples were obtained from each patient</u>.

Samples were lysed in TRIZOL reagent (Invitrogen, Carlsbad CA), and the total RNA fraction was recovered. PolyA-mRNA was purified using a standard protocol. Samples were reverse transcribed to cDNA and conjugated with one of two fluorescent cyan dyes, Cy3 or Cy5. Specifically, normal tissue was labeled with Cy3; and tumor tissue, with Cy5. The labeled cDNA from each tissue sample was hybridized with ESTs immobilized on the microarray. After hybridization, the non-hybridized nucleotides were removed, and a fluorescence scanner was used to detect the dye signal intensity and the degree of hybridization for each array element.

8. **Exhibit D** shows a table of the results obtained using this method for the cDNA encoding SEQ ID NO:19. The first column of the table shows the Cy5/Cy3 ratio. The second column shows the normal lung sample corresponding to the Cy3 measurement. The third column shows the lung tumor sample corresponding to the Cy5 measurement. For example, in the duplicate experiments for donor 7176 (the top two rows of data in Exhibit D), the tissue sample corresponding to the Cy3 value was normal lung tissue from a 72 year-old male, and the tissue sample corresponding to the Cy5 value was lung adenosquamous carcinoma tissue from the same donor. Differential expression between normal and tumor samples on a log 2 scale was 3.9 and 3.8, respectively. I consider significant differential expression (i.e., altered from normal) for the transcript encoding SEQ ID NO:19 in duplicate lung experiments to occur at Cy5/Cy3 ratios greater than 1.5. As can be seen in Exhibit D, this value was exceeded for 26 out of 36 hybridizations (72%) for 18 different sets of matched normal/tumor tissue samples.

Based on these data, it is my opinion that the transcripts for HRM-19 were significantly up-regulated in lung cancer tissue making HRM-19 and the cDNA encoding it very useful in detecting differential expression in lung tumor tissue. It was particularly interesting to note that the transcript encoding HRM-19 was most highly expressed in adenosquamous carcinomas.

9. In conclusion, the literature supports our original characterization of HRM-19 as having homology with <u>C. elegans</u> C16C10 carrier prot in and a specific motif for mitochondrial carrier protein. The microarray expression data fully supports the specific, asserted utility of SEQ ID NOs:19 and 68 in the detection of differential expression associated with lung cancer.

10. I further declare that all statements made herein of my own knowledge are true, and all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date: 09/03/2002.

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